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# CIF150, a Human Cofactor for Transcription Factor IID-Dependent Initiator Function

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**The transcription factor IID (TFIID) complex is highly conserved between the *Drosophila* and mammalian systems. A mammalian homolog has been described for all the *Drosophila* TATA box-binding protein-associated factors (TAFs), with the exception of dTAF$_{II}$150. We previously reported the identification of CIF, an essential cofactor for TFIID-dependent transcription from promoters containing initiator (Inr) elements. Here we describe the molecular cloning of CIF150, the human homolog of dTAF$_{II}$150, and present biochemical evidence that this factor is involved in Inr activity. CIF150 is capable of mediating TFIID-dependent Inr activity in a complementation assay, and a protein fraction lacking Inr activity lacks detectable amounts of CIF150. Despite the striking similarity to dTAF$_{II}$150, CIF150 does not appear to be associated with human TFIID. However, in vitro binding assays revealed a specific and direct interaction between CIF150 and hTAF$_{II}$135. This interaction might be structurally important for the functional interaction between CIF150 and human TFIID, since CIF150 stabilizes TFIID binding to a core promoter.**

The point of entry of RNA polymerase II into the initiation complex is usually defined by sequences located upstream of the coding sequence of the transcribed gene (reviewed in references 17, 21, 32, and 35). These basal promoter elements, which include the TATA box, the initiator (Inr) element, and the more recently defined downstream promoter element (1), are generally not recognized by RNA polymerase II itself (for reviews, see references 17 and 21). In vitro transcription from TATA box-containing promoters by RNA polymerase II can be partially restored by the addition of nuclear fractions containing the general transcription factors IIA (TFIIA), TFIIB, TFIID, TFIIE, TFIIF, TFIIH, and TFIIJ (21). During the first step of preinitiation complex formation, the general transcription factor TFIID recognizes the TATA box and recruits the other general transcription factors and RNA polymerase II to the promoter.

Despite our extensive knowledge of the mechanisms of initiation of TATA box-dependent transcription, initiation from Inr-containing core promoters is not well understood (25; for reviews, see references 17, 21, 27, 32, and 35). An Inr overlaps the transcription start site and is functionally analogous to the TATA box in determining the start site location. Extensive functional analysis of Inr mutants has revealed a loose consensus sequence (PyPyA$_{+1}$NT/APyPy) for Inr activity (8, 13). In promoters containing a TATA box in addition to an Inr, such as the adenovirus major late promoter, both elements act in synergy to define promoter strength. It has been shown that the general transcription factor TFIID, a protein complex containing a TATA box-binding protein (TBP), and several additional factors, known as TAFs (for a review, see reference 32), are required for efficient activity of Inr elements (9, 14, 38). In addition, in vitro binding experiments with highly purified *Drosophila* and human TFIID (hTFIID) complexes have shown that the TFIID-Inr interaction and Inr function are dependent on the presence of specific nucleotides (1, 9, 19, 34). However, in contrast to TATA box-directed transcription, Inr-driven transcription is not dependent on the DNA binding properties of TBP (15). Moreover, trimeric dTBP-dTAF$_{II}$250-dTAF$_{II}$150 has been demonstrated to support Inr activity in an in vitro reconstitution assay, implicating TAFs in mediating Inr activity (31). In this scenario, both dTAF$_{II}$150 and dTAF$_{II}$250 directly bind promoter DNA (30, 31). It is, however, puzzling that an immunopurified hTFIID complex, which has been shown to directly recognize the Inr, lacks a detectable homolog of the dTAF$_{II}$150 protein (2, 9, 11, 14, 38).

Recently, we were able to show by in vitro reconstitution assays that at least one additional cofactor is specifically required to reconstitute Inr-dependent transcription, the cofactor of Inr function (CIF) (10). Inr-dependent transcription was measured as an increase in transcriptional initiation on TATA box-containing promoters in the presence of a consensus or a nonfunctional point-mutated Inr (10). Immunological data obtained with partially purified CIF suggested that a mammalian homolog of dTAF$_{II}$150 might be one subunit of this cofactor. In agreement with this observation, it could be shown that dTAF$_{II}$150 is able to stimulate Inr function (10).

In this paper, we describe the molecular cloning and biochemical characterization of a new human gene and its encoded product, CIF150, the largest subunit of the CIF complex. As expected from previous functional and immunological data, the primary sequence of CIF150 shows clear homology to the sequences of both dTAF$_{II}$150 and TSM-1, an essential protein of the yeast *Saccharomyces cerevisiae*. We demonstrate that despite the striking similarity to dTAF$_{II}$150, CIF150 is not tightly associated with an hTFIID complex but is required for Inr activity on TATA box-containing promoters. In vitro binding assays revealed a specific and direct interaction of CIF150 with hTAF$_{II}$135 and suggested that CIF150 has a role in modulating TFIID binding to the core promoter elements.

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## MATERIALS AND METHODS

**Cloning of CIF150 cDNA.** Oligonucleotides were synthesized based on the mouse-expressed sequence tag W13567 (Washington University) and were used for the isolation of a PCR-amplified internal 1-kb human CIF150 cDNA fragment. Oligonucleotides were derived from this sequence for the isolation of overlapping C-terminal and N-terminal regions of human CIF150 cDNA by use of rapid amplification of cDNA ends in conjunction with a commercially available Marathon-Ready HeLa cell library (Clontech Laboratories, Inc). To ensure correct sequences, two independent clones derived from independent PCRs were sequenced and analyzed. The full-length CIF150 cDNA was cloned into the pET expression vector (Novagen) with N-terminal *Nco*I and C-terminal *Xho*I restriction sites.

**Purification of proteins.** HeLa cell nuclear extracts were prepared as described previously (3). The IA$^-$ protein fraction, which supported TATA box-mediated transcription but not Inr activity, was prepared as described previously (10). The epitope-tagged TFIID complex was isolated from LTRα3 cells by immunoaffinity purification as described previously (38). Recombinant TBP was prepared from *Escherichia coli* as described previously (18).

For Ni affinity purification of CIF, the 0.1 M KCl flowthrough fraction of a DEAE-Sephacel column (10) was applied to a Mono Q (MQ) column and eluted with a linear KCl gradient (40 ml; 0.1 to 1 M). The CIF-containing fractions were pooled and dialyzed against buffer A (20 mM HEPES [pH 7.9], 1 mM EDTA, 3 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 20% glycerol) containing 0.1 M KCl. These fractions were supplemented with imidazole (final concentration, 20 mM in buffer A) and applied to an Ni-nitrilotriacetic acid (NTA)–agarose column (Qiagen). After being washed with 10 column volumes each of 20 mM imidazole and 35 mM imidazole, the bound proteins were eluted with 100 mM imidazole. Protein fractions were tested for CIF activity as described previously (10), and polypeptides were visualized by sodium dodecyl sulfate (SDS)–6% polyacrylamide gel electrophoresis (PAGE) followed by silver staining. To avoid unspecific T7 RNA polymerase transcription in the functional assays, in vitro-translated CIF150 was purified with Ni-NTA–agarose as described above (100 mM imidazole eluate) and concentrated with a Centricon 30 concentrator (Amicon).

**In vitro protein binding studies.** Coupled in vitro transcription and translation reactions were carried out by use of the Promega TNT system with CIF150 cDNA or human TBP (hTBP) cDNA cloned into the pET expression vector, following the instructions provided by the manufacturer (Novagen). The immunopurified hTFIID complex was separated by SDS–6% PAGE (see also silver stain in Fig. 4B) and transferred to nitrocellulose. The protein blot was denatured and subsequently renatured by being washed at 4°C in 1× NET150 (50 mM Tris [pH 7.9], 150 mM NaCl, 0.1% Triton X-100) with 6 M guanidine-HCl, 1× NET150 with 2 M guanidine-HCl, and 1× NET150 with 0.66 M guanidine-HCl for 15 min each. The blot was then incubated for 3 h in NET150 with 5% fat-free dried milk powder, washed with NET150, and incubated overnight at 4°C in NET150 with $2 \times 10^6$ cpm of [$^{35}$S]methionine-labeled protein (CIF150 or hTBP). The blot was finally washed three times with NET150 and exposed to X-ray film.

**In vitro DNA binding experiments.** Electrophoretic mobility shift assays (EMSA) with $Mg^{2+}$-containing agarose gels were performed as previously described (9, 12). The binding mixture contained the DNA probe ($10^4$ cpm) in 30 μl of GL-Buffer (10 mM Tris-HCl [pH 7.9], 10 mM HEPES [pH 7.9], 10% glycerol, 1 mM dithiothreitol, 4 mM $MgCl_2$, 50 mM KCl, 10 mM ammonium sulfate, 100 μg of bovine serum albumin per ml) and was incubated with 3 μl of native CIF alone or in combination with 10 ng of TBP or 10 ng of TFIID (see silver stain in Fig. 2A) for 60 min at 30°C. The probes were prepared by PCR amplification with 5′-end-labeled SP6 primer (Promega) and plasmids J1634 and J1116 (9). The dried agarose gels were exposed to X-ray films and quantitated by use of a PhosphorImager (Molecular Dynamics).

**Western blot analysis.** The protein samples were resolved by SDS–6% PAGE (see also silver stain in Fig. 2A) and transferred to a nitrocellulose membrane. The blot was incubated for 2 h with 5% fat-free dried milk in 50 mM Tris-HCl (pH 7.4)–0.5 M NaCl (antibody dilution buffer). The membrane was then incubated for 2 h each with a 1:500 dilution of a polyclonal antibody directed against the C-terminal part of CIF150 or a 1:5,000 dilution of a monoclonal antibody specific for hTAF250 and hTAF135 (Santa Cruz Biotechnology, Inc.), with biotinylated anti-rabbit–anti-mouse immunoglobulin (BioGenex), and with peroxidase-conjugated streptavidin (BioGenex) in antibody dilution buffer. Between each incubation, the membrane was washed three times with wash buffer (50 mM Tris-HCl [pH 7.4], 0.5 M NaCl, 0.2% Tween 20). After the last incubation, the membrane was washed with wash buffer for 20 min and developed with an ECL kit (Amersham). The polypeptide that cross-reacted with the CIF150 antibody was visualized on Kodak XAR5 film.

**In vitro transcription assays.** In vitro transcription reactions were carried out with templates containing the G-less cassette as described before (5, 10). Plasmid DNAs containing six Sp1 sites and TATA and Inr elements upstream of a 180-bp G-less cassette were constructed with a PCR protocol (10). All plasmid DNAs were amplified in *E. coli* and purified with a standard plasmid preparation protocol (Qiagen). For the complementation assay, 2 μl of the IA$^-$ fraction (4 mg/ml) was preincubated in 30 μl of GL-Buffer for 30 min at 30°C in the presence of 300 ng of DNA template with 1 to 4 μl of CIF activity-containing fractions, followed by the addition of ribonucleoside triphosphates to yield the following final concentrations: 500 μM ATP, 500 μM CTP, and 30 μM [α-$^{32}$P]UTP. The reaction mixture was incubated for an additional 60 min at 30°C, and the $^{32}$P-labeled RNA products were resolved on an 8% polyacrylamide–urea gel and visualized by autoradiography. Signals were quantitated by PhosphorImager analysis.

**Nucleotide sequence accession number.** The CIF150 cDNA sequence reported in this paper has been deposited in the GenBank data bank under accession no. AF026445.

## RESULTS

**Cloning of CIF150 cDNA and its homology to dTAF$_{II}$150.** To characterize CIF activity in greater detail, we sought to isolate the cDNA corresponding to the 150-kDa subunit of this complex. The CIF complex was purified from HeLa cell nuclear extracts (10), and the 150-kDa component was isolated by preparative SDS-PAGE, digested with trypsin, and subjected to microsequence analysis. In agreement with the reported immunological cross-reactivity with dTAF$_{II}$150-specific antibodies (10), one polypeptide was found to be highly homologous to dTAF$_{II}$150 (Fig. 1). In addition, a mouse-expressed sequence tag with distinct homology to dTAF$_{II}$150 was identified in GenBank (accession no. W13567) and used to design an appropriate strategy for cloning of the full-length human cDNA (see Materials and Methods). An open reading frame encoding 1,199 amino acids containing the peptide sequence obtained by microsequencing (Fig. 1) was deduced from a 3,997-bp cDNA sequence by use of two independent clones. Sequence comparisons revealed 53% identity with dTAF$_{II}$150 and 21% identity with the yeast homolog TSM-1 (Fig. 1). These similarities strongly suggest that CIF150 is the human homolog of dTAF$_{II}$150 (30) and of *S. cerevisiae* TSM-1 (20), an essential protein implicated in $G_2$ progression (20, 33).

**Purification of native CIF by Ni affinity chromatography.** The amino acid sequence of CIF150 harbors a distinct stretch of 7 histidine residues near the C terminus (Fig. 1), suggesting Ni affinity chromatography as a possible means for the purification of native CIF. As expected, a 150-kDa protein was specifically enriched (Fig. 2A, cf. lanes 3 and 4). To provide evidence that this protein was indeed CIF150, we performed a functional assay for CIF activity using constructs with six Sp1 binding sites upstream of a TATA box and a downstream wild-type or point-mutated Inr sequence. Figure 3 shows that both a partially purified protein fraction (MQ; Fig. 3, lanes 5 and 6) and the highly purified fraction obtained by Ni affinity chromatography (Fig. 3, lanes 7 to 10) restored initiator activity in a complementation assay with a HeLa cell-derived initiator activity-depleted (IA$^-$) fraction. It should be noted that the IA$^-$ fraction did not lack Sp1 in immunoblot experiments (data not shown). However, a functional assay for CIF activity with constructs without Sp1 binding sites led to the same results, with minor quantitative differences (data not shown) (see also reference 10).

To obtain further evidence that the 150-kDa polypeptide (Fig. 2A, lane 4) is identical to CIF150, we raised polyclonal antibodies against C-terminal portions of recombinant CIF150. In an immunoblot experiment with antibodies directed against CIF150, hTAF$_{II}$250, and hTAF$_{II}$135, both the MQ fraction enriched for CIF activity by conventional chromatography and the Ni affinity-purified CIF fraction yielded a specific 150-kDa band (Fig. 2B, lanes 3 and 4), while no 150-kDa protein was detected by the CIF150-specific antibodies in the IA$^-$ fraction (Fig. 2B, cf. lanes 2 and 9). This data indicates that the 150-kDa polypeptide indeed represents CIF150. In crude nuclear extracts, CIF150 was only detectable when larger amounts of protein were used (Fig. 2B, lane 8), suggesting that CIF150 is a low-abundance protein. Taken together, these results indi-

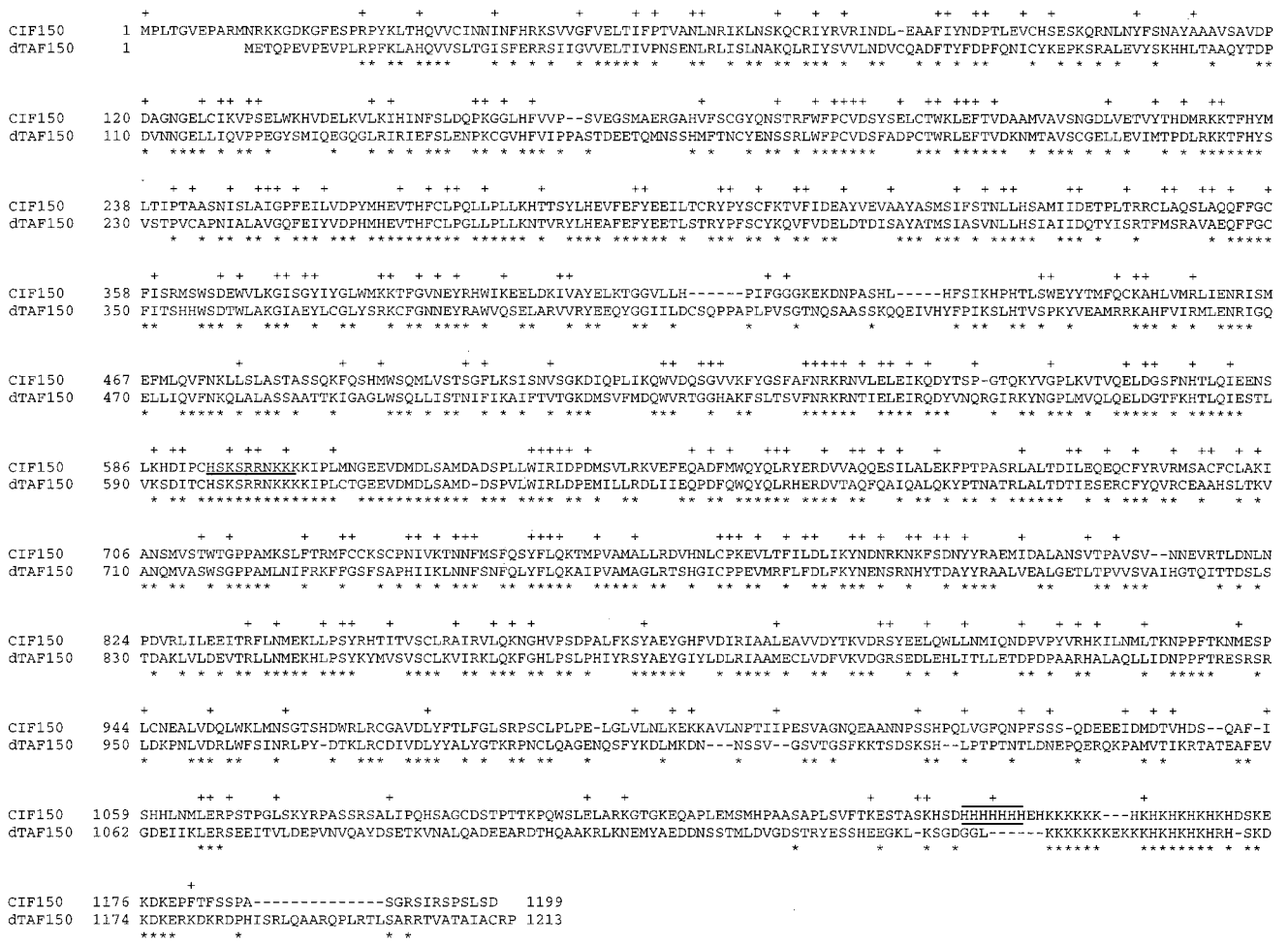


FIG. 1. Alignment of the amino acid sequences of CIF150 and dTAF$_{II}$150. Residues that are shared by both CIF150 and dTAF$_{II}$150 are indicated by asterisks; residues that are identical in CIF150 and the putative yeast homolog TSM-1 are indicated by plus signs. The peptide identified by microsequencing is underlined; the C-terminal histidine stretch is marked by two lines.

cate that Ni affinity chromatography is an efficient method for purifying functional native CIF.

**CIF150 is not associated with immunoaffinity-purified hTFIID.** Since the *Drosophila* homolog of CIF150 is a bona fide TAF (30), we investigated the relationship between the CIF complex and the hTFIID complex. Of the many different hTFIID subcomplexes described, we used the immunoaffinity-purified hTFIID complex derived from the phosphocellulose D fraction (1 M KCl eluate) according to the method of Zhou et al. (38). This purification method leads to at least eight core TAFs (referred to here as bona fide TAFs) that are stably associated with TBP under high-salt conditions (buffer A containing 1 M KCl). To identify whether the immunoaffinity-purified hTFIID complex and the Ni affinity-purified CIF complex have common subunits, we analyzed both complexes by SDS-PAGE (Fig. 2A, lanes 4 and 7). In agreement with previous reports (2, 9, 11, 14, 38), the immunoaffinity-purified hTFIID complex did not contain a 150-kDa polypeptide (Fig. 2A, lane 7). Furthermore, none of the human core TAFs coeluted with CIF150 from the Ni affinity column (Fig. 2A, cf. lanes 4 and 7). However, one polypeptide in the CIF preparation appeared to have a mobility similar to that of hTAF135 (Fig. 2A, lane 4). We therefore tested the comigrating polypeptides by immunoblot analysis with antisera specific for hTAF135 and hTAF250. We were, however, unable to detect these proteins in the CIF preparation with any of these antisera (Fig. 2B, lanes 3 and 4). We were likewise unable to detect CIF150 by immunoblot analysis using highly purified TFIID preparations (data not shown). In agreement with previously reported functional data, TFIID was not depleted in the IA$^-$ protein fraction, which lacks Inr activity (10) (Fig. 2B, lane 2). Taken together, these results indicate that human CIF150 is not a bona fide TAF, despite the homology to dTAF$_{II}$150. On the other hand, the absence of CIF150 in a crude transcription system (IA$^-$) led to a loss of Inr activity (Fig. 3, lanes 3 and 4), indicating that this cofactor is indispensable for TFIID-dependent Inr activity.

**CIF150 mediates Inr activity in vitro.** As shown previously, CIF activity is strictly TFIID dependent and is not detectable in reconstitution experiments with TBP (10). In addition, it was shown that recombinant dTAF150 can partially mediate CIF activity in complementation assays with a protein fraction lacking Inr activity (IA$^-$) (10). Since the Ni affinity-purified CIF preparation still contained several polypeptides next to the CIF150 protein (Fig. 2A, lane 3), we sought to test whether these additional proteins were required to mediate CIF activity using in vitro-translated CIF150 purified by Ni affinity chromatography (see Fig. 4A, lanes 3 and 4). Inr activity was re-

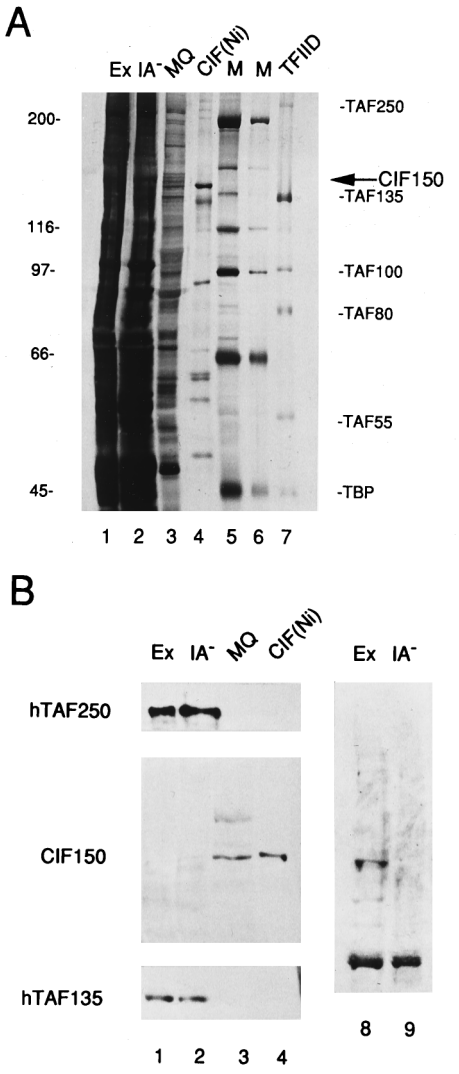


FIG. 2. CIF150 is not associated with hTFIID. (A) Silver stain of an SDS–8% PAGE gel. Lanes 1 and 2 contain 5 μl of HeLa cell nuclear extract and 5 μl of the IA⁻ protein fraction, respectively. Twenty microliters of the loading material (MQ fraction, lane 3) and 20 μl of the eluate from the Ni affinity column (lane 4) were analyzed along with 10 μl of hTFIID (lane 7). The epitope-tagged TFIID complex was isolated from LTRα3 cells by immunoaffinity purification (38). CIF150 protein, which is enriched in the eluate, is indicated by an arrow. The human TAFs are indicated on the right, and sizes of molecular mass standards (lanes 5 and 6) are indicated on the left (in kilodaltons). (B) Antibodies specific for hTAF250, hTAF135, and CIF150 were used in an immunoblot analysis with the same protein fractions and conditions as in panel A (lanes 5 to 7, including hTFIID, are not shown). Lanes 8 and 9 contain four times more protein (20 μl) from HeLa cell nuclear extract and IA⁻ to detect CIF150.

covered, depending on the CIF150 concentration in the described complementation assay, showing that the in vitro-translated protein could substitute for native CIF (Fig. 3, lanes 11 to 14). A mock-treated TNT lysate passed over an Ni affinity column had no effect on transcription (data not shown). This result suggests that CIF activity is not dependent on a larger protein complex. This result is in agreement with the previously determined molecular size for CIF of approximately 200 kDa (10).

**CIF150 interacts specifically with hTAF135.** For $dTAF_{II}150$, a specific interaction with *Drosophila* TBP (dTBP) and $dTAF_{II}250$ has been reported (30). In addition, results with *Drosophila* TFIID components showed that a trimeric complex of dTBP-$dTAF_{II}250$-$dTAF_{II}150$ is minimally required for efficient utilization of the Inr and downstream promoter elements (31). To test whether CIF150 interacts with $hTAF_{II}250$ and/or hTBP, we performed far-Western analysis of highly purified TFIID with radiolabeled CIF150 and radiolabeled TBP as a control (Fig. 4). The two radiolabeled probes and affinity-purified TFIID are shown in Fig. 4A and B, respectively. As expected, TBP interacted specifically with $hTAF_{II}250$ in the far-Western assay (Fig. 4C, lane 1) (6). In contrast, and unlike $dTAF_{II}150$, CIF150 did not interact in this particular in vitro binding assay with either $hTAF_{II}250$ or hTBP but interacted selectively with $hTAF_{II}135$ (Fig. 4C, lane 2). The absence of TBP-CIF150 and $TAF_{II}250$-CIF150 interactions presumably explains why CIF150 does not copurify with TFIID. Interestingly, the domain involved in the interactions with dTBP and $dTAF_{II}250$ has been mapped with coimmunoprecipitation assays to the C terminus of $dTAF_{II}150$ (30), which is poorly conserved in CIF150 (Fig. 1).

**CIF150 stabilizes TFIID binding to the core promoter but does not recognize the Inr itself.** As shown previously, the TFIID complex lacking CIF150 binds with a higher affinity to a promoter containing both a TATA box and an Inr than to a promoter containing a mutant Inr (Fig. 5, cf. lanes 3 and 4) (10, 14). This result confirms the notion that the recognition of the Inr is at least partially mediated by a $TAF_{II}$ component, most likely $TAF_{II}250$ (see below). In order to elucidate the role of CIF150, we analyzed its effect on the TFIID-DNA interaction. Preincubation of immunoaffinity-purified TFIID (Fig. 2A, lane 6) and an Ni affinity-purified CIF complex (Fig. 2A, lane 4) led to a dramatic stabilization of TFIID binding to the promoters (Fig. 5, lanes 5 and 6). TFIID binding was ~10-fold increased in the presence of CIF relative to TFIID binding in the absence of CIF. This stabilization was seen both in the absence and in the presence of the Inr, suggesting that CIF150 is not involved in Inr recognition. Preincubation of TBP together with CIF150 had no effect on DNA binding efficiency (Fig. 5, cf. lanes 11 and 12 with lanes 13 and 14), suggesting that the observed stabilization effect on TFIID binding was mediated by a TAF-CIF150 interaction. The CIF preparation on its own did not show any DNA binding under the same assay conditions (agarose gel electrophoresis EMSA; Fig. 5, lanes 7, 8, 15, and 16), although we were able to detect DNA binding activity of CIF using a conventional polyacrylamide gel-based EMSA technique (data not shown). This DNA binding was Inr independent (data not shown), lending further support to the conclusion that CIF150 is not involved in Inr recognition. These observations are in agreement with previous findings demonstrating that partially purified CIF can restore Inr activity in cooperation with TFIID but not with TBP (10).

## DISCUSSION

The amino acid sequence of CIF150 strongly suggests that CIF150 is the human homolog of dTAF150 and the essential yeast gene *TSM1* (20, 30). A temperature-sensitive mutant of *TSM1* has been shown to lead to a distinct cell cycle phenotype ($G_2$ arrest) (20, 33). This result raises the interesting possibility that CIF150 may also have a role in cell cycle progression, a question that will be addressed in future investigations. Despite the strong homology to $dTAF_{II}150$, CIF150 is not a bona fide TAF in the human system. The fact that $dTAF_{II}150$ but not CIF150 associates with TBP can probably be explained by the low degree of homology at the C terminus, which mediates the interaction of $dTAF_{II}150$ with dTBP and $dTAF_{II}250$ (30). This species-specific difference is reminiscent of the TFIIA-

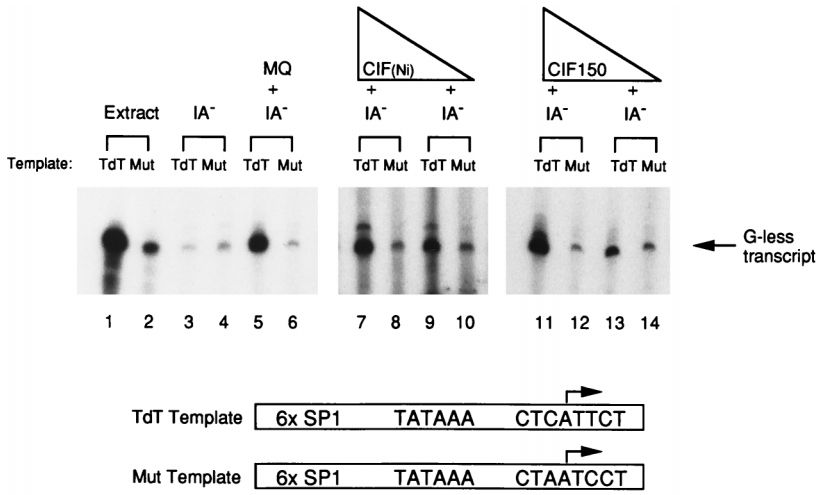


FIG. 3. CIF150 mediates Inr activity in a complementation assay. In vitro transcription assays were performed with plasmids containing six Sp1 sites upstream of a TATA box and the wild-type (odd-numbered lanes) or a point-mutated (even-numbered lanes) Inr element and with HeLa cell nuclear extract (lanes 1 and 2), the IA$^-$ fraction alone (lanes 3 and 4), or the IA$^-$ fraction in combination with the MQ fraction (2 μl; see Fig. 2A, lane 3) enriched for CIF150 (lanes 5 and 6) (10). Lanes 7 to 10 show the IA$^-$ fraction complemented with decreasing amounts (4 μl in lanes 7 and 8 and 2 μl in lanes 9 and 10) of Ni affinity-purified CIF (see Fig. 2A, lane 4), and lanes 11 to 14 show the results of the complementation assay with decreasing amounts (3 μl in lanes 11 and 12 and 1 μl in lanes 13 and 14) of in vitro-translated CIF150 (see Fig. 4A, lanes 3 and 4). The results are representative of three independent experiments.

TFIID interaction. In *Drosophila*, TFIIA and TFIID are tightly associated, whereas in mammalian cells, TFIIA is found as free protein (37). These characteristics may be related to a certain degree of analogy in the functions of TFIIA and dTAF$_{II}$150-CIF150, in that TFIIA contributes to TFIID-TATA interactions and dTAF$_{II}$150-CIF150 stabilizes the binding of TFIID to the Inr.

We have no evidence that CIF150 binds specifically to Inr elements according to the Inr consensus sequence PyPyA$_{+1}$NT/APyPy (8, 13). Our observations are in agreement with previous data showing that components of hTFIID mediate Inr binding. Since the trimeric *Drosophila* complex (dTBP-dTAF$_{II}$250-dTAF$_{II}$150) can substitute for holo-TFIID (31), the most likely candidate for a direct Inr contact seems to be hTAF$_{II}$250. This view is in agreement with the results of UV cross-linking and DNase I footprinting experiments showing direct DNA contacts of dTAF$_{II}$250 (16, 31). Our results suggest that CIF150 can stabilize TFIID-promoter interactions through Inr-independent interactions with DNA and hTAF$_{II}$135. The fact that CIF150 is required to reconstitute Inr-mediated transcription but not TATA box-mediated transcription in functional assays (10, 31; this study) suggests, how-

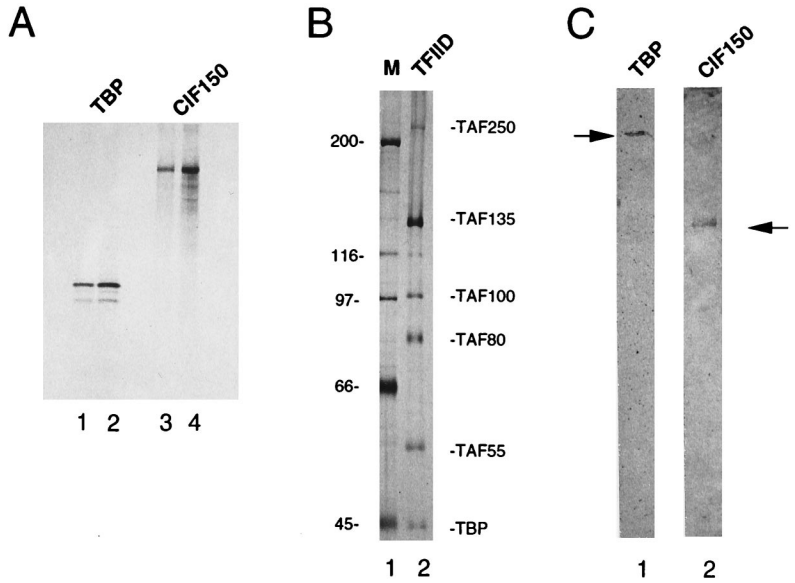


FIG. 4. Direct interaction of CIF150 and hTAF135. (A) Autoradiogram of an SDS–8% PAGE gel loaded with in vitro-translated $^{35}$S-labeled TBP (lanes 1 and 2) and CIF150 (lanes 3 and 4). (B) Silver stain of immunoaffinity-purified hTFIID separated by SDS–8% PAGE. Lane M contains molecular mass standards (kilodaltons). (C) Far-Western analysis of the TFIID preparation shown in panel B (lane 2) with $^{35}$S-labeled TBP (lane 1) and CIF150 (lane 2). Arrows indicate the human TAFs interacting with CIF150 or TBP.

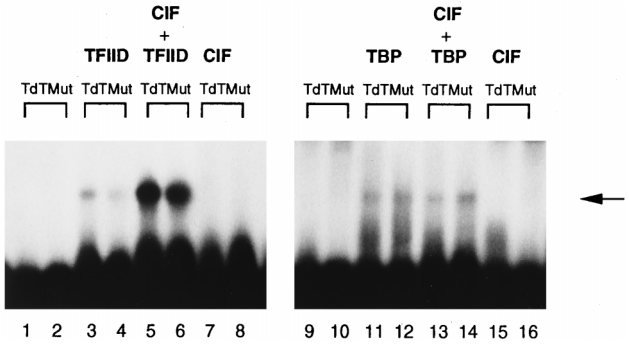


FIG. 5. CIF stabilizes hTFIID but not hTBP binding to the core promoter in a mobility retardation assay. This assay was performed by agarose gel electrophoresis as described previously (12). Probes were derived from plasmids J1634 (wild-type Inr; odd-numbered lanes) and J1116 (point-mutated Inr; even-numbered lanes), and binding conditions were as described before (9). The arrow indicates the specific protein-DNA complex.

ever, that CIF150 has an additional functional role besides stabilizing TFIID binding. Therefore, TFIID-dependent Inr function appears to require at least one additional CIF150-dependent step beyond recognition of the Inr by $hTAF_{II}250$. Whether CIF150 might be involved in recognition of the recently reported downstream promoter element (1) is currently unknown.

It has been demonstrated that the core promoter structure can influence activator function. Thus, the glutamine-rich activation domain of Sp1 preferentially stimulates transcription from Inr-containing core promoters, whereas optimal activation by VP16 requires both TATA and Inr elements (4, 26). An attractive hypothesis explaining this observation, as pointed out by Verrijzer and Tjian (32), is that Sp1 but not VP16 promotes CIF150 recruitment to the core promoter. In this context, it is interesting to note that the human homolog of dTAF110, hTAF135, seems to be the TFIID component that directly interacts with both CIF150 (this study) and Sp1 (7, 28).

Our data clearly demonstrate that the synergistic effect of an Inr in the context of TATA box-containing promoters requires a cofactor, CIF150, that is not needed for TATA box-mediated transcription. It should be noted, however, that there might be additional TFIID- and CIF150-independent mechanisms of Inr-directed transcription. Especially on promoters lacking a functional TATA box, the situation might be more complex, and several proteins besides TFIID have already been implicated in Inr-dependent transcription (22–24, 29, 35, 36; for reviews, see references 17 and 21).

To our knowledge, CIF150 is the first mammalian TFIID cofactor with core promoter element specificity. This novel type of transcription factor may represent a new target for regulatory transcription factors and may also be a key element in elucidating the core promoter heterogeneity found in RNA polymerase II-transcribed genes.

## ACKNOWLEDGMENTS

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